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=> d his

     (FILE 'HOME' ENTERED AT 17:16:11 ON 29 MAR 2007)
     FILE 'REGISTRY' ENTERED AT 17:16:19 ON 29 MAR 2007
L1    698 S CARBONIC ANHYDRASE OR APOCARBONIC ANHYDRASE
          E ALEXA FLUOR 594/CN
L2      2 S E3-4
L3   2126 S FLUORESCENT PROTEIN
L4      0 S L1 AND L3
     FILE 'CA' ENTERED AT 17:20:54 ON 29 MAR 2007
L5  12709 S L1
L6    100 S L2
L7    454 S L3
L8      0 S L5 AND L7
L9      3 S L5 AND L6
L10    12 S L5 AND RATIOMET?
L11  1200 S L5-7 AND ZINC
L12    37 S L5-7 AND ZINC(10A)(FLUOROPHORE OR FLUORESC?)
L13   303 S L5-7(8A) ZINC
L14    15 S L5 AND((ACCEPTOR OR DONOR)(2A)(FLUOROPHORE OR FLUORESC?)OR FRET OR
          (FLUORESC? OR RESONAN?)(3A) ENERGY TRANSFER)
L15    17 S L13 AND(FLUOROPHORE OR FLUORESC?)
L16    35 S (ZINC OR ZN2) AND RATIOMET?(4A)(SENSOR OR DETECTOR OR SENSING OR
          DETECTION OR MEASUR? OR MONITOR?)
L17    86 S L9-10,L12,L14-16
L18    45 S L17 AND PY<2004
L19     7 S L17 NOT L18 AND PATENT/DT
     FILE 'BIOSIS' ENTERED AT 17:33:46 ON 29 MAR 2007
L20    19 S L18
     FILE 'MEDLINE' ENTERED AT 17:34:05 ON 29 MAR 2007
L21     8 S L18
     FILE 'CA, BIOSIS, MEDLINE' ENTERED AT 17:34:47 ON 29 MAR 2007
L22    63 DUP REM L18 L19 L20 L21 (16 DUPLICATES REMOVED)

=> d bib,ab,kwic l22 1-63

L22  ANSWER 7 OF 63  CA  COPYRIGHT 2007 ACS on STN
AN   141:273994  CA
TI   Excitation ratiometric fluorescent biosensor for zinc ion at picomolar
     levels
IN   Thompson, Richard; Cramer, Michele; Fierke, Carol Ann; Zeng, Hui Hui;
     Bozym, Rebecca
PA   USA
SO   U.S. Pat. Appl. Publ., 19 pp., which
PI   US 2004185518        A1    20040923    US 2003-673409         20030930
PRAI US 2002-414657P      P     20021001
     US 2002-416515P      P     20021008
AB   A highly selective and sensitive carbonic anhydrase-based method for
     measurement of zinc ion by an excitation ratiometric format based on
     resonance energy transfer: i.e., where the zinc ion level is transduced
     as the ratio of fluorescence intensities excited at two different
     excitation wavelengths, is provided.  The method can be used very well
     in a fluorescence microscopy format.  A detection limit of about 10 pM
     in zinc buffered systems, a ten to one thousand-fold improvement on the
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Fura indicators (which respond to Ca and Mg as well), and a one hundred thousand-fold improvement on the recently described FuraZin-1 is achieved. The L198C variant of human apocarbonic anhydrase II conjugated with Alexa Fluor 594 at the introduced cysteinyl residue and dapoxyl sulfonamide were used to quantitate zinc ion.

L22 ANSWER 15 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 139:32777 CA
TI Excitation **ratiometric fluorescent** biosensor for **zinc** ion at picomolar levels
AU Thompson, Richard B.; Cramer, Michele L.; Bozym, Rebecca; Fierke, Carol A.
CS Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, 21201, USA
SO Journal of Biomedical Optics (**2002**), 7(4), 555-560
AB Zinc is a metal ion of increasing significance in several biomedical fields, including neuroscience, immunol., reproductive biol., and cancer. **Fluorescent** indicators have added greatly to our understanding of the biol. of several metal ions, most notably calcium. Despite substantial efforts, only recently have zinc indicators been developed which are sufficiently selective for use in the complex intra- and extracellular milieus, and which are capable of quantifying the free zinc levels with some degree of reliability. However, these indicators (such as FuraZin-1 and Newport Green DCF) have only modest sensitivity, and there is growing evidence that significantly lower levels of free zinc may be biol. relevant in some instances. We have adapted the peerless selectivity and sensitivity of a carbonic anhydrase-based indicator system to an excitation **ratiometric** format based on **resonance energy transfer**: i.e., where the **zinc** ion level is transduced as the ratio of **fluorescence** intensities excited at two different excitation wavelengths, which is preferred for **fluorescence** microscopy. The system exhibits more than a 60% increase in the ratio of intensity excited at 365 nm to that excited at 546 nm (emission obsd. at 617 nm). The detection limit is about 10 pM in zinc buffered systems, a 10-1000-fold improvement on the Fura indicators (which respond to Ca and Mg as well), and a 10000-fold improvement on the recently described FuraZin-1.

L22 ANSWER 21 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 135:148901 CA
TI Protein compactness measured by **fluorescence resonance energy transfer**. Human carbonic anhydrase II is considerably expanded by the interaction of GroEL
AU Hammarstrom, Per; Persson, Malin; Carlsson, Uno
CS IFM-Department of Chemistry, Linkoping University, Linkoping, SE-581 83, Swed.
SO Journal of Biological Chemistry (**2001**), 276(24), 21765-21775
AB Nine human carbonic anhydrase II (HCA II) single-Cys mutants were labeled with 5-(2-iodoacetylaminoethylamino)naphthalene-1-sulfonic acid (1,5-IAEDANS), an efficient **acceptor** of Trp **fluorescence** in **fluorescence resonance energy transfer** (**FRET**). The ratio between the fluorescence intensity of the 5-(2-acetylaminoethylamino)naphthalene-1-sulfonic acid (AEDANS) moiety excited at 295 nm (Trp absorption) and 350 nm (direct AEDANS absorption) was used to est. the av. distances between the 7 Trp

residues in human carbonic anhydrase II (HCA II) and the AEDANS label. Guanidine-HCl denaturation of the HCA II variants was also performed to obtain a curve that reflected the compactness of the protein at various stages of unfolding, which could serve as a scale for the expansion of the protein. This approach was developed in this study and was used to est. the compactness of HCA II during heat denaturation and interaction with GroEL. It was found that thermally induced unfolding of HCA II proceeded only to the molten globule state. Reaching this state was sufficient to allow HCA II to bind to GroEL, and the vol. of the molten globule intermediate increased □2.2-fold compared with that of the native state. GroEL-bound HCA II expanded to a vol. 3- to 4-fold that of the native state (to □117,000 Å3), which correlated well with a stretched and loosened-up HCA II mol. in an enlarged GroEL cavity. Recently, the authors found that HCA II binding causes such an inflation of the GroEL mol., and this probably represents the mechanism by which GroEL actively stretches its protein substrates apart, thereby facilitating rearrangement of misfolded structure.

L22 ANSWER 23 OF 63 MEDLINE on STN
AN 2001156252 MEDLINE
TI Enhanced fluorescence resonance energy transfer between spectral variants of green **fluorescent** protein through **zinc**-site engineering.
AU Jensen K K; Martini L; Schwartz T W
CS Laboratory for Molecular Pharmacology, Department of Pharmacology, The Panum Institute, University of Copenhagen, Copenhagen, Denmark.
SO Biochemistry, **(2001 Jan 30)** Vol. 40, No. 4, pp. 938-45.
AB Although spectral variants of GFP should in theory be suited for fluorescence resonance energy transfer (FRET) and therefore suited for studies of protein-protein interactions, the unfavorable location of the fluorophore 15 A deep inside the GFP molecule has especially impaired this application. Here, metal-ion site engineering around the dimerization interface known from the X-ray structure of GFP is applied to the cyan and the yellow spectral variant of GFP to stabilize the heterodimeric form of these molecules and thereby increase FRET signaling. The FRET signal, determined as the ratio between the maximal emission for the yellow variant, 530 nm, and the cyan variant, 475 nm, during excitation of the cyan variant at 433 nm was increased up to 8-10-fold in the presence of 10(-4) M ZnCl2 by engineering of two symmetric metal-ion sites being either bidentate or tridentate. A similar increase in FRET signaling was however obtained in a pair of molecules in which a single bidentate metal-ion site was generated by introducing a zinc-binding residue in each of the two spectral variants of GFP and therefore creating an obligate heterodimeric pair. It is concluded that FRET signaling between spectral variants of GFP can be increased by stabilizing dimer formation and especially by favoring heterodimer formation in this case performed by metal-ion site engineering.

L22 ANSWER 24 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 136:196318 CA
TI **Fluorescence**-based biosensing of **zinc** using carbonic anhydrase
AU Fierke, Carol A.; Thompson, Richard B.
CS Departments of Chemistry and Biochemistry, University of Michigan, Ann Arbor, MI, USA

SO BioMetals (**2001**), 14(3-4), 205-222
AB A review. Measurement of free zinc levels and imaging of zinc fluxes remains tech. difficult due to low levels and the presence of interfering cations such as Mg and Ca. We have developed a series of **fluorescent zinc** indicators based on the superb sensitivity and selectivity of a protein, human apo-carbonic anhydrase II, for Zn(II). These indicators transduce the level of free zinc as changes in intensity, wavelength ratio, lifetime, and/or anisotropy; the latter three approaches permit quant. imaging of zinc levels in the microscope. A unique attribute of sensors incorporating biol. macromols. as transducers is their capability for modification by site-directed mutagenesis. Thus we have produced variants of carbonic anhydrase with improved affinity for zinc, altered selectivity, and enhanced binding kinetics, all of which are difficult to modify in small mol. indicators.

L22 ANSWER 25 OF 63 BIOSIS on STN
AN 2002:168755 BIOSIS
TI Chemistry of **zinc**(II) **fluorophore** sensors.
AU Kimura, Eiichi [Reprint author]; Aoki, Shin
CS Department of Medicinal Chemistry, Faculty of Medicine, Hiroshima University, Minami-ku, Hiroshima, 734-8551, Japan ekimura@hiroshima-u.ac.jp
SO Biometals, (**September-December, 2001**) Vol. 14, No. 3-4, pp. 191-204.
AB The biological role of the zinc(II) ion has been recognized in DNA and RNA synthesis, apoptosis, gene expression, or protein structure and function. Therefore, development of useful zinc(II) sensors has recently been attracting much interest. Chemistry for selective and efficient detection of trace Zn2+ is a central issue. Recently, various types of **zinc-fluorophores** are emerging, comprising bio-inspired aromatic sulfonamide derivatives, **zinc**-finger peptides attached to **fluorescent** dyes, or **fluorophore**-pendant macrocyclic polyamines. The chemical principles, properties and limitations of these Zn2+-fluorophores are discussed.

L22 ANSWER 26 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 136:337167 CA
TI **Ratiometric fluorescence** imaging of free Zn2+ in brain
AU Thompson, Richard B.; Suh, Sang Won; Frederickson, Christopher J.
CS University of Maryland School of Medicine, USA
SO Proceedings of SPIE-The International Society for Optical Engineering (**2001**), 4255(Clinical Diagnostic Systems), 88-93
AB Recently, the function of zinc in the axonal boutons of hippocampal neurons has come under increased scrutiny as evidence has emerged of a putative role for this metal ion in neural damage following insults such as ischemia, blunt force trauma, and seizure. Indeed, the nonpathol. role of free zinc in the brain remains cryptic after more than 40 yr. The authors have used a biosensing approach to det. free **zinc** ion concns. by **fluorescence** lifetime, intensity, intensity ratio, or anisotropy changes caused by binding of zinc to variants of a protein, apocarbonic anhydrase II (apo-CA). This approach permits real time measurement of zinc down to picomolar levels, with no perceptible interference from other divalent metal ions abundant in serum and tissue, such as calcium and magnesium. Recently, we used apo-CA

together with a **fluorescent** ligand whose binding is metal-dependent to obtain the first **fluorescence** micrographs of **zinc** release from a rat hippocampus model in response to elec. stimulus. In our view, elucidation of the zinc fluxes in neural tissue ultimately requires quantitation, as in the case of calcium. Recent results will be shown.

L22 ANSWER 31 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 133:116873 CA
TI **Zinc** biosensing with multiphoton excitation using carbonic anhydrase and improved **fluorophores**
AU Thompson, Richard B.; Maliwal, Badri P.; Zeng, Hui-Hui
CS Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, 21201, USA
SO Journal of Biomedical Optics (**2000**), 5(1), 17-22
AB Previously, we had shown that the **zinc**-dependent binding of certain **fluorescent** aryl sulfonamide inhibitors could be used with apocarbonic anhydrase II to transduce the level of free **zinc** as a change in the **fluorescence** of the inhibitor. While inhibitors such as dansylamide, ABD-M, and ABD-N made possible quantitation of free zinc in the picomolar range with high selectivity, they have only modest absorbance which limits their utility. We describe here the synthesis and properties of two new probes, Dapoxyl sulfonamide and BTCS, and their use in zinc biosensing. Dapoxyl sulfonamide exhibits a dramatic increase and blue shift in its emission upon binding to holocarbonic anhydrase II, as well as a 20-fold increase in lifetime: it is thus well suited for quantitating free Zn(II) down to picomolar ranges. The anisotropy of BTCS increases fivefold upon binding to the holoprotein, making this probe well suited for anisotropy-based detn. of zinc. BTCS and ABD-N are efficiently excited with two photon excitation using 1.5 ps pulses from a titanium sapphire laser, and exhibit the increased zinc-dependent anisotropy response anticipated on the basis of photoselection.

L22 ANSWER 33 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 131:208273 CA
TI Enzyme-based fluorescence biosensor for chemical analysis
IN Thompson, Richard B.; Patchan, Marcia W.; Ge, Zhengfang
PA USA
SO U.S., 25 pp.

PI	**US 5952236**	**A**	**19990914**	**US 1996-736904**	**19961025**
	US 6225127	**B1**	**20010501**	**US 1999-270308**	**19990315**
	US 6197258	**B1**	**20010306**	**US 1999-273303**	**19990319**
PRAI	US 1995-5879P	P	19951026		

AB This invention generally relates to the detection, detn., and quantitation of certain ions and small mols. involving the quenching of a fluorescent label attached to a macromol., often due to **fluorescence energy transfer** to a colored inhibitor or certain metal ions bound to the macromol.

L22 ANSWER 35 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 133:116938 CA
TI Improved response of a fluorescence-based metal ion biosensor using engineered carbonic anhydrase variants

AU Thompson, Richard B.; Zeng, Hui-Hui; Loetz, Michele; McCall, Keith; Fierke, Carol A.
CS Dep. Biochem. Mol. Biol., Univ. of Maryland, Sch. Med., Baltimore, MD, USA
SO Proceedings of SPIE-The International Society for Optical Engineering (**1999**), 3858(Advanced Materials and Optical Systems for Chemical and Biological Detection), 161-166
AB The response time of biosensors which reversibly bind an analyte such as a metal ion is necessarily limited by the kinetics with which the biosensor transducer binds the analyte. In the case of the carbonic anhydrase-based biosensor we have developed the binding kinetics are rather slow, with the wild type human enzyme exhibiting an assocn. rate const. ten thousand-fold slower than diffusion-controlled. By designed and combinatorial means the transducer may be mutagenized to achieve nearly diffusion-controlled assocn. rate consts., with commensurate improvement in response. In addn., a variant of apocarbonic anhydrase was immobilized on quartz, and is shown to response rapidly to changes in free copper ion in the picomolar range.

L22 ANSWER 36 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 131:239944 CA
TI Fluorescence-based sensing of transition metal ions by a carbonic anhydrase transducer with a tethered fluorophore
AU Thompson, Richard B.; Maliwal, Badri P.; Fierke, Carol A.
CS Dep. Biochem. Mol. Biol., Univ. of Maryland School of Medicine, Baltimore, MD, USA
SO Proceedings of SPIE-The International Society for Optical Engineering (**1999**), 3602(Advances in Fluorescence Sensing Technology IV), 85-92
AB We have demonstrated that free metal ions such as Zn(II) can be detd. by fluorescence anisotropy (polarization) using an apometalloenzyme, carbonic anhydrase II, and a fluorescent aryl sulfonamide inhibitor of the enzyme whose affinity for the enzyme is metal-dependent. We felt that attaching the fluorescent aryl sulfonamide to the protein would provide a similar response, while avoiding problems of disproportionation of the inhibitor and protein. In fact a tethered aryl sulfonamide ABD-T gave very good results: Zn(II) and Cu(II) at picomolar levels and Co(II), Cd(II), and Ni(II) at nanomolar levels can all be detd. by changes in fluorescence intensity, anisotropy, and lifetime using visible excitation sources. Implications of these results are discussed.

L22 ANSWER 37 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 131:239947 CA
TI Improved **fluorophores** for **zinc** biosensing using carbonic anhydrase
AU Thompson, Richard B.; Maliwal, Badri P.; Zeng, Hui-Hui
CS School of Medicine, Univ. of Maryland School of Medicine, Baltimore, MD, USA
SO Proceedings of SPIE-The International Society for Optical Engineering (**1999**), 3603(Systems and Technologies for Clinical Diagnostics and Drug Discovery II), 14-22
AB Previously, we had shown that the **zinc**-dependent binding of certain **fluorescent** aryl sulfonamide inhibitors could be used with apo-carbonic anhydrase II to transduce the level of free **zinc** as a change in the

fluorescence of the inhibitor. While inhibitors such as dansylamide, ABD-M, and ABD-N made possible quantitation of free zinc in the picomolar range with high selectivity, they have only modest absorbance which limits their utility. We describe here the synthesis and properties of two new probes, Dapoxyl sulfonamide and BTCS, and their use in zinc biosensing. Dapoxyl sulfonamide exhibits a dramatic increase and blue shift in its emission upon binding to holo-carbonic anhydrase II, as well as a twenty-fold increase in lifetime: it is thus well suited for quantitating free Zn(II) down to picomolar ranges. The anisotropy of BTCS increases five-fold binding to the holoprotein making this probe well suited for anisotropy-based detn. of zinc.

L22 ANSWER 39 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 129:51549 CA
TI Expanded Dynamic Range of Free **Zinc** Ion Determination by **Fluorescence** Anisotropy
AU Thompson, Richard B.; Maliwal, Badri P.; Fierke, Carol A.
CS Department of Biochemistry and Molecular Biology, University of Maryland School of Medicine, Baltimore, MD, 21201, USA
SO Analytical Chemistry (**1998**), 70(9), 1749-1754
AB We demonstrate that by use of a biosensor approach employing wild type human apocarbonic anhydrase II and a newly synthesized **fluorescent** ligand, ABD-M, free Zn(II) may be detd. in soln. at concns. in the picomolar range with good accuracy by **fluorescence** anisotropy. **Fluorescence** anisotropy enjoys the same freedom from artifact as wavelength **ratiometric** approaches widely used for detg. metal ions in soln. such as Ca(II). In addn., we demonstrate that anisotropy-based detns. exhibit an important advantage, a broad dynamic range, which has not been demonstrated for wavelength **ratiometric** approaches. In particular, by judicious choice of excitation and emission wavelengths, the concn. range over which Zn(II) may be detd. accurately can be increased by approx. 2 orders of magnitude. As ABD-M also exhibits significant changes in excitation and emission spectra as well as lifetime upon binding to the active-site Zn(II) in holocarbonic anhydrase, it should also be useful for wavelength **ratiometric** and lifetime-based detns.

L22 ANSWER 44 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 126:41814 CA
TI A Novel Biomimetic **Zinc**(II)-**Fluorophore**, Dansylamidoethyl-Pendant Macrocyclic Tetraamine 1,4,7,10-Tetraazacyclododecane (Cyclen)
AU Koike, Tohru; Watanabe, Tomohiko; Aoki, Shin; Kimura, Eiichi; Shiro, Motoo
CS School of Medicine, Hiroshima University, Hiroshima, 734, Japan
SO Journal of the American Chemical Society (**1996**), 118(50), 12696-12703
AB From the chem. principle of carbonic anhydrase (CA)-arom. Sulfonamide inhibitor interaction, a dansylamidoethyl-pendant cyclen (1-(2-(5-(dimethylamino)-1-naphthalenesulfonamido)ethyl)-1,4,7,10-tetrazacyclododecane, HL) was synthesized as a novel type of Zn(II)-**fluorophore**. The new ligand HL forms very stable complexes (ML) with Zn (II), Cd(II), and Cu(II) at physiol. pH. The potentiometric and spectrophotometric pH-titrn. study disclosed the 1:1 metal(II) complexes stability consts. log K(ML) (= log([ML]/[M][L])) to be 20.8 ± 0.1 for

ZnL, 19.1 ± 0.1 for CdL, and >30 for CuL. The cryst. Zn(II) complex ZnL was isolated from aq. soln. at pH 7. The x-ray crystal study of ZnL disclosed a five-coordinate, distorted square-pyramidal structure with the deprotonated dansylamide N- coordinating at the apical site. Crystals of the monoperchlorate salt of ZnL ($C_{22}H_{35}N_6O_6SClZn$) are orthorhombic, space group Pna21 with a 23.777(3), b 12.744(5), c 9.092 (3) Å, Z = 4, R = 0.032, and Rw = 0.047. The Zn(II) complex shows a max. UV absorption band (λmax) at 323 nm (ε 5360) at 25° in aq. soln. The **fluorescent** max. and the quantum yield (Φ) of ZnL vary with the solvent: at 528 nm (Φ = 0.11) in H_2O, 496 nm (0.53) in MeOH, 489 nm (0.60) in EtOH, and 484 nm (0.44) in MeCN. Demetalation of ZnL with excess amt. of EDTA yielded the metal-free ligand HL, which in pH 7.3 aq. soln. has an excitation and a weak emission **fluorescence** at 330 nm (ε 4950) and 555 nm (Φ = 0.03), resp. The Cu(II) ion, to the contrary, completely quenches the **fluorescence**. The cryst. Cu(II) complex CuL (λmax 306 nm, ε 7630 in H_2O) was isolated as its monoperchlorate salt. The Zn(II)-dependent **fluorescence** with 5 μM HL at pH 7.3 is quant. responsive to 0.1-5 μM concn. of Zn(II), which is unaffected by the presence of mM concn. of biol. important metal ions such as Na+, K+, Ca2+, and Mg2+. The new ligand HL forms a far more stable 1:1 Zn(II) complex than any previous Zn(II) **fluorophore** and is evaluated as a new Zn(II) **fluorophore**.

L22 ANSWER 45 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 125:189766 CA
TI Structure-Based Design of a Sulfonamide Probe for **Fluorescence** Anisotropy Detection of **Zinc** with a Carbonic Anhydrase-Based Biosensor
AU Elbaum, Daniel; Nair, Satish K.; Patchan, Marcia W.; Thompson, Richard B.; Christianson, David W.
CS Department of Chemistry, University of Pennsylvania, Philadelphia, PA, 19104-6323, USA
SO Journal of the American Chemical Society (**1996**), 118(35), 8381-8387
AB Given the avid and selective metal binding properties of naturally-occurring metalloproteins, it is possible to exploit these systems in the development of novel sensors, i.e., "biosensors", for the detection of trace quantities of metal ions. Here, we exploit the high affinity of human carbonic anhydrase II (CAII) for zinc in the detection of nanomolar concns. of this metal ion by fluorescence anisotropy using a fluorescein-derivatized arylsulfonamide probe, 4-aminosulfonyl[1-(4-N-(5-fluoresceinylthioureido)butyl)]benzamide (3). This probe was designed through an iterative, structure-based approach and was demonstrated to bind tightly only to the zinc-bound holoenzyme (Kd = 2.3 nM) and not the metal-free apoenzyme. Furthermore, the probe exhibits anisotropy that is proportional to the concn. of bound zinc, and this behavior can be exploited in the detection of zinc in the 10-1000 nM range. Strategies for the structure-based design of improved CAII-based metal ion biosensors are considered in view of these results.

L22 ANSWER 51 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 124:163395 CA
TI Site-specific mutants of carbonic anhydrase for **fluorescence energy transfer**-based metal ion biosensing
AU Thompson, Richard B.; Ge, Zhengfeng; Patchan, Marcia W.; Kiefer, Laura

L.; Fierke, Carol A.
CS School Medicine, University Maryland, Baltimore, MD, 21201, USA
SO Proceedings of SPIE-The International Society for Optical Engineering (**1995**), 2508(Chemical, Biochemical, and Environmental Fiber Sensors VII), 136-44
AB In order to gain wavelength and analyte flexibility, we have recently altered the transduction approach of our fluorescence-based biosensor. Briefly, binding of metal ions such as zinc to the active site of carbonic anhydrase is transduced by metal-dependent binding of a colored inhibitor to a fluorescent deriv. of the enzyme; in the absence of metal the inhibitor does not bind and the label fluorescence is unquenched, but at higher metal concns. the inhibitor binds, energy transfer occurs with moderate efficiency and the fluorescent label exhibits reduced intensity and lifetime. Inasmuch as Forster energy transfer is distance dependent the position of the fluorescent label on the surface of the enzyme has some impact on the performance of the sensor. We designed, produced, and expressed site-selective mutants of carbonic anhydrase which could be unambiguously derivatized with suitable **fluorescent** labels, and which gave much improved responses to **zinc** ion compared with randomly derivatized wild type enzyme.

L22 ANSWER 52 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 122:285866 CA
TI Lifetime-based **fluorescence energy transfer** biosensing of **zinc**
AU Thompson, Richard B.; Patchan, Marcia W.
CS Dep. Biol. Chem., Univ. Maryland Sch. Med., Baltimore, MD, 21201, USA
SO Analytical Biochemistry (**1995**), 227(1), 123-8
AB A new type of **fluorescence** transduction method for detg. **zinc** in soln. is described. The approach is based upon **energy transfer** from a **fluorescent** label on an enzyme, human carbonic anhydrase II, to a colored inhibitor which binds to zinc in the enzyme active site. If zinc is present in soln., it binds to the apoenzyme, which in turn permits the inhibitor to bind to the enzyme; the inhibitor is thus in close proximity to the label on the enzyme and thereby quenches the label's **fluorescence** by Forster **energy transfer** with a concomitant redn. of its lifetime, which is quantitated by phase fluorometry.

L22 ANSWER 55 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 118:115727 CA
TI Enzyme-based fiber optic zinc biosensor
AU Thompson, Richard B.; Jones, Eric R.
CS Sch. Med., Univ. Maryland, Baltimore, MD, 21201, USA
SO Analytical Chemistry (**1993**), 65(6), 730-4
AB A **fluorescence**-based fiber optic biosensor capable of detecting **zinc**(II) at nanomolar concns. is described. The sensor transduces the specific recognition of the ion by an enzyme (carbonic anhydrase) as a change in the **fluorescence** of an inhibitor which binds to the **zinc** in the active site. The concn. of metal ion is proportional to the ratio of fluorescence intensity at two wavelengths, corresponding to the emission from found and free inhibitor. Sensing of zinc may be performed through a single optical fiber.

L22 ANSWER 61 OF 63 CA COPYRIGHT 2007 ACS on STN

AN 83:39328 CA
TI Effect of **zinc** on the activity and **fluorescence** of carbonic anhydrase holoenzymes
AU Hesketh, T. Robin; Flanagan, Michael T.
CS Natl. Inst. Med. Res., London, UK
SO Biochemical Journal (**1975**), 147(1), 37-43
AB Enhancement of the **fluorescence** of human carbonic anhydrase (EC 4.2.1.1) (I) B haloenzyme by Zn2+ was correlated with the inhibition by Zn2+ of p-nitrophenyl acetate hydrolysis by I. Acetate or Cl- increased the affinity for Zn2+, suggesting that the inhibitory Zn2+-binding site was within the region of I which undergoes an anion-induced conformational change. Zn2+ also enhanced the **fluorescence** of bovine I and the C isoenzyme of human I, indicating that the binding site was not a thiol group. Zn2+ induced a major conformational change in the C, but not the B, isoenzyme. On the basis of model compd. studies the effect of Zn2+ was attributed to either direct chelation to a tryptophan residue or chelation near a tryptophan residue neutralizing an adjacent protonated amino group.

L22 ANSWER 63 OF 63 CA COPYRIGHT 2007 ACS on STN
AN 68:19060 CA
TI Combination of bovine carbonic anhydrase with a fluorescent sulfonamide
AU Chen, Raymond F.; Kernohan, John C.
CS Natl. Heart Inst., Bethesda, MD, USA
SO Journal of Biological Chemistry (**1967**), 242(24), 5813-23
AB Bovine erythrocyte carbonic anhydrase (I) forms a highly fluorescent complex with 5-(dimethylamino)naphthalene-1-sulfonamide (DNSA) (II). The binding, studied either by enhancement of ligand fluorescence or by the quenching of protein uv fluorescence, shows that only 1 mole of II is bound/mole of protein; the dissocn. const. at pH 7.4 is 2.5 × 10-3M. The fluorescence of free II in water has peak emission at 580 mμ and a quantum yield of only 0.055, but bound II has an emission max. at 468 mμ and a yield of 0.84. Arguments are presented to explain the large emission blue shift on the basis that the binding site is extremely hydrophobic and that the SO2NH2 group of the ligand loses a proton upon binding to the enzyme. The binding appears specifically to involve the sulfonamide site known to exist in I; several other "fluorescent probe" compds. showed no evidence of binding to the enzyme. Calcn. of the energy transfer efficiency indicated that 85% of the photons absorbed by the 7 tryptophan residues are transferred to the single bound II mol. The transfer efficiency is much higher than hitherto observed for a protein having only one 5-(dimethylamino)naphthalene-1-sulfonyl group. Although the diam. of the protein is □51 A., the bound II group is probably within the crit. transfer distance of 21.3 A. of all the tryptophans. The effective av. distance between II and tryptophan was 16 A. The fluorescence properties of the complex were quite different from those of a conjugate prepared by reaction of 5-(dimethylamino) naphthalene-1-sulfonyl chloride with I. The sulfonamide-binding site and the tryptophan residues may be in the interior of the protein. The tryptophan fluorescence of the protein was 73% quenched by the binding of 1 II mol. Although large, this degree of quenching was less than the overall efficiency of energy transfer of photons absorbed by the protein. This result indicates that the fluorescence efficiencies of the 7 tryptophans are different, and that II is bound in such a way that

energy transfer occurs with greater probability from those tryptophan residues which are relatively less fluorescent. I inhibits the esterase activity of I as tested with the substrate, p-nitrophenyl acetate.

=> log y
STN INTERNATIONAL LOGOFF AT 17:35:42 ON 29 MAR 2007